ISOLATION AND PURIFICATION OF PHENOL OXIDASE FROM THE LEAVES OF THE COTTON PLANT

T. S. Yunusov and P. Kh. Yuldashev

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Continuing an investigation of the phenol oxidase of the cotton plant [1], we have isolated and purified this enzyme.

The freeze-dried leaves (23 g) obtained from 120 g of fresh leaves collected in the period of pinching out the buds (July, 1972) were extracted with 1150 ml of 0.01 M citric-phosphate buffer with pH 7.8-8.0 containing 1% of ascorbic acid and 50 g of Kapron powder. The mixture was filtered through a layer of gauze and then through a layer of filter paper. The green-pink extract was dialyzed against 0.001 M phosphate buffer, pH 7.8-8.0, until the wash waters no longer absorbed at 260 nm. The dialyzate was deposited on a column (4 × 40 cm) of DEAEC I equilibrated with the same buffer. The rate of flow of the solution during the deposition of the protein on the column was 60 ml/h. The protein was sorbed on DEAEC II in the form of a narrow band. Elution was performed in a gradient of potassium chloride. The rate of elution was 50 ml/h. The results of the fractionation are given in Fig. 1.

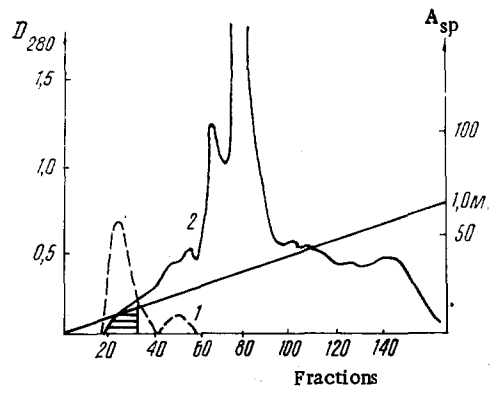

Fig. 1

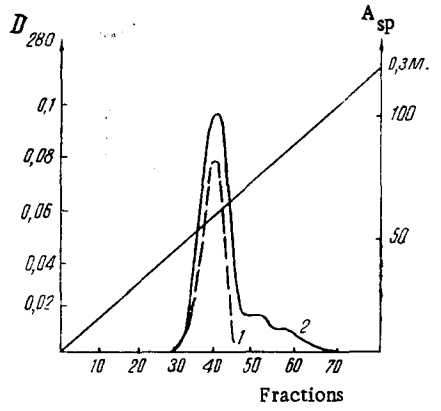

Fig. 2

Fig. 1. Fractionation of the total protein on DEAEC I: 1) A_{sp}; 2) D_{280}.

Fig. 2. Rechromatography of the active fraction of the protein after DEAEC I on DEAEC II: 1) A_{sp}; 2) D_{280}.

TABLE 1

Purification stage	Volume of solution, ml	A_{tot}	A_{sp}	Amt. protein, mg	Yield of enzyme, %		Degree of purification of the enzyme
					prot.	activ.	
Extract after dialysis	1450	5,5	14	580	100	100	0
DEAEC I	110	7,7	60	14,3	2,5	16	4,3
DEAEC II	50	10	80	6	1	7	5,7

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The fraction with the highest specific phenol oxidase activity was again dialyzed against 0.001 M phosphate buffer, pH 7.8-8.0, and was deposited on a column (1 × 27 cm) of DEAEC equilibrated with the same buffer. Elution was performed in a potassium chloride gradient at the rate of 12 ml/h. The results of rechromatography are shown in Fig. 2. The protein obtained, when rechromatographed on DEAEC, was eluted as a symmetrical peak coinciding with the peak of the activity of the enzyme. The results on the isolation of the phenol oxidase are summarized in the table. The concentration of protein was determined by the biuret method and also from the absorption at 280 nm. The activity (A) was measured by the colorimetric method.

In an investigation of the phenol oxidase by the method of unestablished equilibrium [2] on an MOM 3170 ultracentrifuge at 22,000 and 8,000 rpm it was seen that the protein was homogeneous and had a molecular weight of 11,700. For the ultracentrifugation, 5 mg of protein was dissolved in 1 ml of 0.1 M phosphate buffer, pH 7.8.

Thus, a phenol oxidase with a molecular weight of 11,700 has been isolated in the homogeneous state from the leaves of the cotton plant.

LITERATURE CITED

1. T. S. Yunusov and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 446 (1973).
2. W. J. Archibald, J. Phys. and Coll. Chem., 51, 1204 (1947).